Table 1. Immunohistochemical localization of proteins during spermiation

Protein (*GENE*)	Summary	Localization[a] *Before*	*Stage VII*	*Stage VIII*	Cell expression	Possible function	Refs.*
14-3-3 family	- family containing 7 isoforms expressed in most tissues with >200 binding partners - regulatory proteins which bind to various signalling molecules - 14-3-3β binds TESK1 [122] - 14-3-3θ expressed with a shortened 3'UTR in Sertoli cells [123], and localized to the apical ES [124] - knockdown of 14-3-3θ in primary Sertoli cells disrupts N-Cadherin and ZO-1 localization [124] - function of 14-3-3 family in testis reviewed in [125]	14-3-3θ: localized with spermatids at all stages [124]	14-3-3θ: D [124]	14-3-3θ: almost undetectable [124]	14-3-3θ: GC > SC [124]	ES Signalling - early	124, 126
Actin (*ACT*)	- endogenous protein to eukaryote cells - individual G-actin subunits assemble into F-actin polymers - polymerisation of actin subunits can form microfilaments in the cytoplasm providing mechanical support, signal transduction, formation of specialised intracellular structures for endocytosis and phagocytosis. - cytochalasin D used to disrupt actin filaments in the testis [127]	all stages	D, V, R	A	SC + GC	ES TBC Adhesion - entire	24, 128
Afadin myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to 4 (*MLLT4*)	- nectin- and actin-filament binding protein that connects nectin to the actin cytoskeleton - regulates cell-cell adhesions downstream of Ras activation - localization is dependent upon Nectin 2 in testis [28] - component of the N-cadherin/β-catenin/α-actinin/actin complex at ES [28]	all stages [28]	NA, likely D, V [28]	NA	NA, likely SC [28]	ES	[28]
Amphiphysin 1 (*AMPH*)	- linker between clathrin and dynamin; recruits dynamin to clathrin - implicated in phosphatidylserine-dependent phagocytosis in Sertoli cells [129] and TBC formation [99] - *Amph*$^{-/-}$ mice demonstrate reduced TBC number and impaired spermiation [99] - associated with Rac1 and Cdc42 signalling pathways [97]	with eST from VI [99]	V [99]	Absent [99]	SC [129]	TBC	99, 129-131

Aquaporin (*AQP*)	- family of integral membrane proteins that form pores within the membrane and transport small molecules - possible roles in recycling surplus cytoplasmic components of elongated spermatids [109]	AQP11: in association with eST [109]	AQP11: residual eST cytoplasm [109]	AQP11: residual body [109]	AQP11: eST [109]	Potential role in spermatid cytoplasm remodelling	[109]
Arp2/3 complex actin related protein 2/3 complex	- seven subunit complex, each of which is expressed in the testis [60] - couples nucleation and branching of actin filaments with nucleation promoting factor proteins, including n-WASP and cortactin (reviewed in [132]) - disrupted Arp2/3 expression in a model of spermiation failure [133] - inhibition of WASP activation, and Arp2/3 branching resulted in mis-orientation of spermatids *in vivo* [26] - component of n-WASP/amphiphysin/actin complex in testis [97]	absent	V,R [26]	Absent [26]	SC	TBC	[24, 133-135]
Cadherin family	- large family of transmembrane proteins with important roles in cellular adhesion - family includes cadherins, protocadherins, desmogleins, and desmocollins - contain extracellular "cadherin repeats" with Ca^{2+} binding domains - N-Cadherin expressed within the Sertoli cell [136] - Testicular review [137]. Conjecture over the presence of apical ES cadherin content based on subtype localization during spermiation - PanCad antibody which recognises a conserved Cadherin domain has been used to immunolocalise cadherins in the testis [37]	NA	PanCad around elongated spermatid [37, 53]	PanCad around elongated spermatid [37]	SC + GC	Unknown Cadherin *may* be present: Adhesion-entire	[53, 29]
β Catenin (*CTNN*)	- catenins (including α, β, γ and δ) are a small family of proteins - β-catenin is a subunit of the cadherin complex and has implications as a co-factor in Wnt signalling - binds actin/xyxin/WASP complex by co-IP [101]	localized near clusters of eSTs [67]	A [37]	A [37, 67]	SC [105]	Adhesion - entire	[37, 67]
Cathepsin (*CTS*)	- family of proteases activated at low pH; generally function as proteases in lysosomes - CTSL is a secretory product of Sertoli cells [138, 139] and is stimulated by FSH [140] - *Furless* mice which contain a point mutation in CTSL have increased tubule atrophy, reduced germ cells, but fertile [141] suggesting redundancy with other cathepsin family members	CTSD: associated with the acrosome [142] CTSK: Sertoli cell stalk, granular [143] CTSL: associated with eST [143]	CTSK: Sertoli cell stalk, granular [143] CTSL: V, D [138, 143]	CTSK: Sertoli cell stalk, granular [143] CTSL: associated with eST [143]	CTSD: SC [144] CTSK: SC [143] CTSL: SC [145, 146]	Protease – entire	

CDC42 cell division cycle 42 (GTP binding protein, 25kDa) **(*CDC42*)**	- small GTPase of the Rho subfamily - activity stimulated by TGFβ in primary Sertoli cells [147] - expression of dominant negative mutant blocked endocytosis and junction turnover in primary Sertoli cells [147] - directly binds and activates n-WASP for filopodia formation [148]	all stages [147]	A [126, 147]	A [126, 147]	SC + GC [147]	ES, Signalling - entire	126, 147
Clathrin **(*CLHC*)**	- facilitates the formation of small vesicles from the cell membrane - requires adapter and partner proteins for recruitment to plasma membrane and endocytotic function - siRNA to CLHC in primary Sertoli cells reduces junction endocytosis [149]	NA	V, F [22]	NA	SC + GC	TBC [22]	EM [22]
Cofilin 1 ***(CFL1)***	- calcium dependent F-actin-depolymerising protein - phosphorylated by LIMK [150], TESK1 [151] and TESK2 [152], contributing to actin cytoskeletal remodelling in cell lines - both cofilin isoforms expressed in the testis, although CFL1 is predominant [25] - may form part of a RhoB/ROCK/LIMK/CFL axis in the testis [153]	NA	V, R [25]	NA [25]	unknown, likely SC	TBC	[25]
Connexin 43 ***(GJA1)***	- component of Gap Junctions - Sertoli cell specific knockout has spermatogonial arrest [154], reduced gap junctions, but functional tight junctions [155]	associated with spermatids as they penetrate SC crypt [156]	A [154, 156]	A, absent late VIII [156]	SC	Membrane channels	154, 156
Cortactin ***(CTTN)***	- class II action nucleation-promoting factor which activates the Arp2/3 complex [132] for actin branching - reduction in cortactin levels [135] and increased tyrosine phosphorylation following anti-androgen or –estrogen treatment [134]	NA	V, R [22]	D [22]	SC	TBC, Adhesion - entire	22, 133-135
CSK c-src tyrosine kinase **(*CSK*)**	- tyrosine kinase that phosphorylates and controls the activity of pp60src - immunoprecipitates with cadherin/catenin proteins [157]	Yes [157]	D [67, 157, 158]	D [67, 157, 158]	SC (+ GC?)	ES Signalling - entire	67, 157, 158
Desmoglein **(*DSG*)**	- desomosomal cadherins are calcium binding transmembrane proteins - four family members; -1, -2 and -4 expressed in the testis [159] - DSG-2 co-IP with c-SRC in the testis [159]	Yes [67]	V [67]	V but removed in late VIII [67]	SC [159] and sperm [67]	ES Adhesion - early	67

Dynamin ***(DNM)***	- family of three GTP binding proteins associated with microtubules - implicated in formation of a variety of actin based cellular events, including endocytosis - DNM2 implicated in phosphatidylserine-dependent phagocytosis in Sertoli cells [129] and TBC formation [99] - DNM3 tubularises cellular membranes [24]	DNM2: concentrated in regions where the acrosome is related to the nucleus [24] DNM3: No [24]	DNM2: V [99, 129] DNM3: V, R [24]	No	SC + GC	DNM2: TBC DNM3: TBC	DNM2: localization [99, 129, 160] DNM3: localization [22, 24]
EEA1 early endosome antigen 1 **(*EEA1*)**	- contains FYVE domain which binds PtdIns3P with high specificity and affinity in early endosomes - early endosomes are cellular compartments that receive and sort endocytosed materials for degradation or recycling	NA	V, F [29]	NA	Not reported, likely SC [29]	TBC	NA
EPS8 epidermal growth factor receptor pathway substrate 8 **(*EPS8*)**	- participates in actin-based motility by capping barbed ends of actin filaments [161] - transduces signals from Ras to Rac [162] - RNAi in primary Sertoli cells leads to mislocalization of TJ protein components [163] - *Eps8*$^{-/-}$ fertile [162]	from stage V [163]	V [163]	V, low levels [163]	SC apical and basal sites [164]	Adhesion - early TBC	Expression [163, 164] Localization [163]
ERK extracellular signal-regulated kinase	- widely expressed protein kinase intracellular signalling molecules - active ERK significantly increased in tubules during spermiation [126] - reviewed in [165]	NA	ERK and p-ERK in apical cytoplasm [126]	ERK and p-ERK: strong localization in association with spermatids [126].	SC	Signalling - entire	[126] Many others
Espin **(*ESPN*)**	- links actin filaments into hexagonal bundles [166] - espin mutant “Jerker males” fertile [167] - FSH stimulates the recruitment of espin into the ES in primary SC [168]	Yes, from step 8 [166]	V, D [35, 166]	Disappears early VIII [166]	SC apical and basal ES	ES	[35, 36, 58, 166]
Fimbrin **(*PLS1*)**	- actin bundling protein - may be involved in cross-linking ctin filaments in the ES	NA. Likely from step 8 [169]	NA	NA	NA	ES [169]	[169]
FAK focal adhesion kinase 1/protein tyrosine kinase 2 **(*PTK2*)**	- focal adhesion related protein kinase involved in cellular adhesion to extracellular matrices and cell spreading - phosphorylated in response to integrin engagement, acts as a central hub of focal adhesion function - remains associated with spermatid up until the point of disengagement, together with integrins [55] - remains localized to spermatids that fail to release [55]	Yes, from step 8 [56]	FAK: basal sites only pFAK-Tyr^{397}: D, V pFAk-Tyr^{576}: D, V [56]	FAK: basal sites only pFAK-Tyr^{397} and pFAk-Tyr^{576} around spermatid [56], shifts to D in VIII [55]	SC	ES Signalling - entire	[55, 56]

FYN FYN oncogene related to SRC **(*FYN*)**	- protein tyrosine kinase, with an important role in cell proliferation and differentiation - interacts with PI3K - fyn knockout mice fertile, although transient reduction in testis weight during development and degenerating germ cells observed with ES abnormalities [170]	Yes [170]	NA [170]	Sertoli cell 'stalk' pattern, and around spermatid head [170]	SC [170]	ES Signalling. - entire	170
GALECTIN lectin, galactoside-binding, soluble **(*LGALS*)**	- family of carbohydrate binding proteins - LGALS3 stimulated by FSH in cultured primary Sertoli cells [171]	LGALS1: yes [172] LGALS3: yes [171]	LGALS1: D, V [76] LGALS3: NA [171]	LGALS1: D, V [76] LGALS3: NA [171]	LGALS1: SC > GC [172] LGALS3: SC > GC [171]	LGALS1: ES, Adhesion - entire	
KEAP1 kelch-like ECH-associated protein 1 **(*KEAP1*)**	- binds nuclear transcription factor Nrf2 and retains it in the cytoplasm, inhibiting nuclear function - binds myosin-VIIa at the ES [173]	Yes [173]	Localized around spermatid head [173]	NA	SC	ES Adhesion - early	173
Integrin **(*ITG*)**	- family of transmembrane receptors that mediate cellular attachment and cell signalling - bind to fibronectin, vitronectin, collagen and laminin - consists of heterodimer between α and β subunit - α6β1 predominant heterodimer in testis [55, 126], likely mediating attachment to laminin on spermatid [59] - α6β1 remains associated with spermatid up until the point of disengagement, together with FAK [55]	Yes [57, 58, 174]	β1: eST head [53, 57, 58, 174] α6: eST head [58, 174]	β1: D [53, 57, 58, 174] α6: D [58, 174]	SC	ES Adhesion - entire	37, 53, 55
ILK Integrin linked-kinase **(*ILK*)**	- interacts with β-integrins; acts as a receptor kinase regulating integrin-induced signal transduction - IP with β1 integrin in testis [53] - removed from spermiation machinery prior to FAK and integrins [55]	from step 8 spermatids [53]	D, F [53]	Absent [37]	SC	ES Adhesion - early	53
JAM junctional adhesion molecule **(*JAM*)**	- JAM family members are typically associated with tight junctions in epithelial tissues, although JAM3 is associated with adherens junctions [175] - JAM2 and JAM3 colocalise around spermatids in the testis and may form an interaction between Sertoli cells and spermatids [175] - JAM3 is essential for the differentiation of rST to eST in mice [175] - JAM3 co-IP with CAR [176] and Pals1 [105] in testis	JAM2 and JAM3: From rST polarisation onwards [175]	JAM2: NA JAM3: D – spermatid [175]	NA	JAM2: SC [175] JAM3: eST > SC [175]	ES Adhesion – entire (spermatid)	175

Laminin family	- binds integrin and MMPs by co-IP [104] - various laminin forms expressed in the testis; laminin-α3, -β3, -γ3 co-IP with β1-integrin in the testis [59]	γ3: expressed by eST from XIII [104]	α3: D – spermatid [59] γ3: D – spermatid [59, 104]	α3: D – spermatid [59] γ3: D – spermatid [59, 104]	eST	ES Adhesion – entire (spermatid)	[104]
MEK MAP Kinase / ERK kinase	- widely expressed kinases that belong to the MAP kinase kinase family - phosphorylate ERK to mediate downstream signals	prominent in germ cell cytoplasm [126] not reported at developing ES	V, D [126]	Weak staining; V, D [126]	PSC >> SC	Signalling - entire	[126]
MMP Matrix metallo-protease	- family of zinc-dependent proteases capable of degrading extracellular matrices - MMP-2 and TIMP-2 stimulated by FSH in Sertoli cell [177] - MMP-2 stimulated by retinol and retinoic acid in Sertoli cell [178] - MT1-MMP and MMP-2 co-IP with laminin components [104] - Pro- and active-forms of MMPs present in whole testis isolates [104] - MMP-9 also expressed [104] - numerous MMPs expressed in fetal testis [179]	MT1-MMP: throughout spermatogenesis [104, 177] MMP-2: associated with PSC and rST [104] TIMP2: associated with PSC and rST [104]	MT1-MMP: V, D [104, 177] MMP-2: V, D [104] TIMP2: V, D [104]	MT1-MMP: V, D [104, 177] MMP-2: V, D [104] TIMP2: V, D [104]	SC + GC	Protease - entire	
MYOSIN (*MYO7A*)	- superfamily of motor proteins responsible for actin based motility - Myh9 expressed in Sertoli cells and phosphorylated with FSH signalling [180] - MyosinVIIa expressed is association with the basal and apical ES [173, 181]	at site of ES with spermatid at all stages [173]	MYO7a: V, D [173, 181]	NA	SC [173]	ES Adhesion - early	[173, 181]
NECTIN poliovirus receptor-related 2 **(*PVRL*)**	- Nectins are a family of Ca^{2+}-independent cell-cell adhesion molecules, which homo- and hetero-dimerise and cause cell-cell adhesion - nectin-based cell-cell adhesion induces activation of Cdc42 and Rac proteins, which regulate adherens junction formation by reorganizing the actin cytoskeleton - *Nectin 2*$^{-/-}$ and *Nectin 3*$^{-/-}$ mice have male specific infertility [182, 183] - Nectins localize at distal end of TBC, suggestive of TBC internalization [29]	NA	V, D [182, 183]	NA	PVRL2: SC PVRL3: GC	ES Adhesion - early	Localization [28, 29, 184]

PAK p21 activated kinase **(*PAK*)**	- Serine/Threonine kinase signal transducers activated by direct binding by GTPase dependent (Rac and Cdc42) and independent (PKB and PDK1) mechanisms - coIP with c-Src, PDK1 and PKB in the testis [102]	localized with eST from stage VI [102]	V, D [102]	Reduced; V, D[102]	PAK1/2: Sertoli cell [102]	Signalling - early	102
PAR6 partitioning defective complex **(*PARD*)**	- Par proteins help establish cell polarity and may be central in the establishment or maintenance of TJs - Par3 and 6 are expressed in the testis; Par6 coIP with JAM3 and Pals1 [105] - Par6 may mediate spermatid polarity in association with the Sertoli cell [175]	localized with eSTs at all stages [105]	V, D [105]	Absent [105]	SC > GC [105]	ES Signalling - early	105
Paxillin **(PXN)**	- focal adhesion associated protein implicated in cell signalling - coIP with vinculin and FAK [53]	diffuse SC localization	V, A [53]	D [67]	SC > rST [67]	TBC [53], Signalling-entire	53, 67
PI-3 kinase phosphoinositide-3-kinase (***PIK3***)	- PI3Ks are a family of intracellular signal transducing enzymes that phosphorylate phosphatidylinositol molecules to regulate downstream signalling - coIP with β1-integrin, FAK, p-FAK Tyr^{397}, paxillin, p130Cas, vinculin, and Gelsolin in testis lysates [102] - inactive p110β transgenic mice exhibit male specific sub-fertility [185]	p85α: from step 8 spermatid [102] p110β: NA	p85α: V, D [102] p110β: NA	p85α: V, D [102] p110β: NA	p85α: SC + GC [102] p110β: SC + GC [185]	ES Signalling - entire	102, 185
PI(4,5)P2 Phosphatidylinositol 4,5-bisphosphate	- membrane bound phospholipid; acts as a substrate for signal transduction - phosphorylated by PI-3 kinases, and dephosphorylated by PTEN	localized around eST [186]	NA	NA	NA	ES (other localization unknown)	186
Protein Kinase B v-akt murine thymoma viral oncogene **(*AKT*)**	- family of serine/threonine kinases, also known as Akt - regulator of numerous cell survival pathways - coIP with PAK, Actin, Vimentin and α-tubulin in the testis [102] - phosphorylation stimulated by IL-1β [187], FSH [188-190], cAMP [188, 190] and IGF-1 [190, 191] in SC	PKB and p-PKB-Ser^{473} in association with ES throughout [102]	PKB and p-PKB-Ser^{473}: V, D [102]	PKB and p-PKB-Ser^{473}: D, reduced in late VIII [102]	SC + GC [102, 192]	ES Signalling - early	193, 194
Phospholipase C **(*PLC*)**	- family of phospholipid lipases - cleave PIP2 into DAG and IP3; thought to stimulate cellular Ca^{2+} and PKC activity - PLCβ1 gene disruption causes infertility [195]	PLCγ: Absent prior to spermiation [186]	PLCγ: V, D [186]	NA	PLCγ: SC [186]	Signalling - early (localization after ES removal not described)	186

PTEN phosphatase and tensin homolog (***PTEN***)	- phosphatase of PIP3 (PI3K pathway) and several actin related proteins, including FAK[397] - stimulated by FSH in Sertoli cells [196] - Co-IP with Vimentin, α-Tubulin, Actin, PAK1,2 in seminiferous tubules [48]	all stages [102]	V, D [102]	D [102]	SC + GC [102, 197]	ES. Signalling - entire	
RAB RAB4A, member RAS oncogene family RAB8A, member RAS oncogene family RAB12, member RAS oncogene family RAB14, member RAS oncogene family	- small RAS superfamily members with GTP binding and signalling activity; context and isoforms specific roles - RAB8A interacts with Myosin Vb and co-localises with EHD1 and EHD4 [198] - RAB4A IP with α- and β-catenin, actin, vimentin, α- and β-tubulin, and protein kinase C [199] - RAB8A IP with actin, vimentin, α-tubulin and β-tubulin in SC [200] - RAB13: IP with actin, vinculin, espin, and weakly with vimentin and γ-catenin [201] - Rab5 a marker of early endosomes in isolated Sertoli cells [202]	RAB4A: with eST from at least stage IV [199] RAB8A: stage XII spermatids [200] RAB12: associated with eST [203] RAB13: eST at all stages except VIII [201]	RAB4A: associated with eST [199] RAB12: in association with eST [203] RAB13: associated with eST [201]	RAB4A: associated with eST [199] Rab8a: not present [200] RAB12: not present [203] RAB13: not present [201]	Rab4A: SC > GC [199] RAB8a: SC + GC [200] RAB12: SC [203] RAB13: SC + GC [201]	RAB4A: Signalling-entire [199] RAB12; ES, Signalling early RAB13: ES, Signalling – early RAB8a: inconclusive	RAB4A: expression and localization [199] RAB8B expression and localization [200]
SRC v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (***SRC/p60src/c-src***)	- tyrosine kinase whose activity is inhibited by CSK - Androgen Receptor binding to SRC may modulate ERK signalling pathways [204] - administration of c-Src inhibitor induced the loss spermatocytes and round spermatids from the seminiferous epithelium [157] - immunoprecipitations with cadherin/catenin proteins [157]	with round spermatids from step 9, increasing and maximal at VIII[205]	D[67]	D [67, 205]	SC	Signalling - entire	[67, 205, 206]
TESTIN testis derived transcript (3 LIM domains) (***TESS***)	- interacts with a variety of cytoskeletal proteins including zyxin, VASP and actin [207] - loss of testin from focal adhesions results in loss of actin stress fibres in HeLa cells [208] - SC expression modulated by germ cells [209] - knockout viable, with increased incidence of tumours. NA fertility [210]	with eSTs throughout, although most prominent at VIII [211]	D and predominantly V [211, 212]	V, but absent prior to release [211, 212]	SC [209]	Adhesion - entire	Localization [211]
Vinculin (***VCL***)	- membrane-cytoskeletal protein in focal adhesion plaques involved in the linkage of integrin to the actin cytoskeleton - recruitment to ES is FSH dependent [168] - coIP with p-FAK-Tyr[397] [56] and Paxillin [56]	with round spermatids from step 8 [36]	V, D, A [36, 37]	Absent [36, 37]	SC > GC [56, 168]	ES Adhesion - early	[37, 53, 56, 67, 126, 169, 213]

n-WASP Wiskott-Aldrich syndrome-like **(*WASL*)**	- actin nucleation promoting factor; participates in actin branching in combination with cortactin (reviewed [132]) - inhibition of WASP activation, and Arp2/3 branching results in mis-orientation of spermatids *in vivo* [26] - knockout of n-WASP interacting partner exhibits male sterility [98] - phosphorylated by FAK [214], Rac1 and PIP2 [215]	No	V [22, 26]	Absent [22]	SC, basal and apical sites	TBC	
Zyxin (*ZYX*)	- Zinc binding phosphoprotein; concentrates at focal adhesions - IP with N-cadherin/b-catenin/α-actinin/actin complex [101]	associated with eST [101]	associated with ES [101]	NA	GC > SC [101]	ES	

* References can be found in the references list of the main manuscript found here: http://www.landesbioscience.com/journals/spermatogenesis/article/14525/

[a] NA = no information available d = dorsal curvature, v = ventral curvature, A = diffuse staining of apical Sertoli cell cytoplasm, F = focal dots of staining, R = radial spoke-like pattern in inner ventral curvature.

[b] Our view of the likely localization of the protein in the spermiation machinery based on available micrographs of the immunohistochemical localization. For the purpose of this analysis, the spermiation machinery is subdivided as follows:

ES : present at the SC-spermatid interface from step 8-19. Thus this protein remains associated with the ES structure and/or adhesion domain throughout spermiogenesis
Adhesion; early: Describes an adhesion or adhesion-associated protein that likely participates in adhesion early in the spermiation process, when the ES structure is present. Often, such proteins become more obvious between eST and SC near the time that spermiation is initiated (eg. Stages V-VII). Such proteins are not observed in mid-late stage VIII tubules prior to disengagement
Adhesion; entire: Describes an adhesion or adhesion-associated protein that likely participates in adhesion throughout the entire spermiation process, from the initiation in stage VII until disengagement late in stage VIII.
TBC: Describes a protein that is likely present at or within TBCs, based on a characteristic localization at the inner curvature of the spermatid head in stage VII
Signalling (early, or entire): Describes a signalling protein that is either present a) early in the spermiation process, when the ES structure is present but is not present just prior to disengagement in stage VIII(early); or b) is present throughout the entire spermiation process, from the initiation in stage VII until disengagement late in stage VIII
Protease (early, or entire): Describes a protease that is either present a) early in the spermiation process, when the ES structure is present but is not present just prior to disengagement in stage VIII(early); or b) is present throughout the entire spermiation process, from the initiation in stage VII until disengagement late in stage VIII